AMENDMENTS TO THE SPECIFICATION

Please amend page 1, between lines 3 and 4 to include the following paragraph:

This application claims priority of Application No. 2000-374145, filed in Japan on December 8, 2000 under 35 U.S.C. §119.

Please amend the paragraph at page 1, lines 10-19 as follows:

Cranial nervous diseases including Creutzfeldt-Jakob disease (hereinafter referred to as "CJD" for short), scrappy disease of sheep, transmissible encephalopathy of mink and the like develop after a long incubation period. They are mainly characterized by spongiform change of brain tissue and amyloid spot (kuru spot), that are ~~almost~~ mostly localized in the nerve system, and progressively ~~aggravate~~ worsen and lead to death. Although the cause of the diseases has not been fully clarified, the so called "prion hypothesis" which assumes that the diseases are not caused by infectious pathogen such as a virus, but are caused by deposition of abnormal type prion protein, is now believed by most of the researchers. These diseases are diagnosed by pathological analysis of thin section of brain tissue.

Please amend the paragraph at page 2, lines 9-20 as follows:

In human, although variations in a part of the amino acid sequence (primary structure) of the prion protein among individuals have been reported, there is no difference between the amino acid sequences of prion protein in CJD patients and normal individuals. Therefore, it is thought that deposition of the abnormal prion protein is not because of the amino acid sequence, but because of the difference in stereostructure. Therefore, the conventional anti-prion antibodies which recognize the primary amino acid sequence of prion cannot distinguish the abnormal type prion from the normal type prion. Further, since the amino acid sequence is well conserved between animals, it is presumed that ~~antigenecity~~ antigenicity of prion is low. Thus, there is a demand for the production of an anti-abnormal type prion antibody using an immunogen keeping the stereostructure thereof, in which the ~~antigenecity~~ antigenicity of the immunogen is increased ~~increased, is demanded~~.

Please amend the paragraph starting at page 2, line 26 and ending at page 3, line 19 as follows:

It is thought that a monoclonal antibody which can distinguish the abnormal type prion from the normal type prion may be obtained by immunizing an animal with the abnormal type prion, obtaining monoclonal antibodies by a conventional method, and by screening a monoclonal antibody which reacts with the abnormal type prion but does not react with the normal type prion. However, not only because of the fact that there are no differences in the amino acid sequence of the normal and abnormal types, but also because of the low species specificity, it is difficult to induce an antibody, especially an antibody which can distinguish the abnormal type prion from the normal type prion. Further, abnormal type prion is a protein which is difficult to obtain in a sufficient amount for use as an immunogen. The frequency of the diseases yielding abnormal type prion is low, and the facilities which can deal with the abnormal type prion are limited because it is a strong pathogen. Because of <u>the above</u> ~~these~~, it is difficult to obtain the abnormal type prion in a large amount. Further, to use the abnormal type prion protein as an immunogen, it is necessary to purify the protein to some degree. However, the protein is insolubilized in the purification step, and the insolubilized protein is not suitable as an immunogen. To use as an immunogen, the protein is solubilized using a modifier or the like. However, it is difficult to ensure that the stereostructure unique to the

abnormal type be kept during the solubilization step. Because of these reasons, it is difficult to prepare a monoclonal antibody which can distinguish the abnormal type prion from the normal type prion.

Please amend the paragraph starting at page 3, line 24 and ending at page 4, line 19 as follows:

That is, the present invention provides an anti-abnormal type prion monoclonal antibody which reacts with abnormal type prion but does not substantially react with normal type prion by antigen-antibody reaction, or an antigen-binding fragment thereof. The present invention also provides a hybridoma which produces the monoclonal antibody according to the present invention. The present invention further provides a method for measuring abnormal type prion by an immunoassay utilizing the antigen-antibody reaction between the monoclonal antibody according to the present invention and an abnormal type prion. The present invention still further provides an immunoassay kit for carrying out the immunoassay of the present invention, comprising the monoclonal antibody or the antigen-binding fragment thereof according to the present invention. The present invention still further provides process for producing the anti-abnormal type prion monoclonal antibody according to the present

invention, comprising immunizing an animal with an immunogen including a peptide containing a plurality of regions in said abnormal type prion, which regions are discontinuous with each other in the primary amino acid sequence of said abnormal type prion; preparing hybridomas originated from antibody-producing cells of the immunized animal; screening a hybridoma which produces an anti-abnormal type prion monoclonal antibody which reacts with the abnormal type prion but does not substantially react with the normal type prion by antigen-antibody reaction; and recovering said anti-abnormal type prion monoclonal antibody from the hybridoma selected by the screening. The present invention still further provides an immunogen used in the above-mentioned process for producing the monoclonal antibody of the present invention.

Please amend the paragraph at page 5, lines 5-17 as follows:

The monoclonal antibody according to the present invention reacts with the abnormal type prion by antigen-antibody reaction but does not substantially ~~reacts~~ react with the normal type prion. The term "does not substantially react" means that the immunological reactivity with the normal type prion is lower than the immunological reactivity with the abnormal type prion to a discernable degree. Thus, even if a monoclonal antibody has a

cross-reactivity with the normal type prion, in case where the affinity to the normal type prion is lower than the affinity to the abnormal type prion to a discernable degree, the monoclonal antibody is included within the definition of "does not substantially react with the normal type prion", and is included in the scope of the present invention. Needless to say, a monoclonal antibody which does not have a cross-reactivity with the normal type prion, that is, a monoclonal antibody which reacts with the abnormal type prion but does not react with the normal type prion is preferred.

Please amend the paragraph at page 6, lines 9-16 as follows:

The present invention also provides antigen-binding fragments of the above-described monoclonal antibody according to the present invention. The term "antigen-binding fragment" herein ~~means~~ means a fragment such as Fab fragment or $F(ab')_2$ fragment of the antibody, which exhibits antigen-binding property of the antibody. These fragments may easily be obtained by cleaving the monoclonal antibody of the present invention with papain or pepsin according to a conventional method. These antigen-antibody fragments may be used in the

immunoassays described below equally as the monoclonal antibody of the present invention.

Please amend the paragraph starting at page 6, line 17 and ending at page 7, line 22 as follows:

As mentioned above, it is difficult to prepare a monoclonal antibody which reacts with the abnormal type prion but does not substantially react with the normal type prion by using the abnormal type prion as an immunogen. To solve this problem, the present inventors originally presumed the stereostructure of the abnormal type prion. Fig. 1 shows the stereostructure of the normal prion (PrP^{C} model), the stereostructure of the abnormal type prion presumed by Korth et al. (PrP^{SC} model 1, C. Korth et al., Nature 390:74-77, 1997), and the stereostructure of the abnormal type prion (PrP^{SC} model 2) presumed by the present inventors. The stereostructure of the abnormal type prion, which was presumed by the present inventors, contains more β sheet structures than in the stereostructure presumed by Korth et al. The grounds of this presumption are as follows: From the results of CD spectrum and FT-IR, it is said that during the process of changing from the normal type to the abnormal type, α helix structure is decreased from 40% to 30%, and β structure is increased from very little to 45%. According to the calculation based on the stereostructure (123-231a.a.) by NMR, except for

the very flexible repeating sequence at the N-terminal of which structure cannot be determined, the secondary structure of the normal type contains 25% of α helix and 3.4% of β structure (assuming that the full length is 231 amino acid residues). Assuming that the contribution to the content of α helix structure by the N-terminal region which cannot be included in the calculation is about 15%, according to the model of Korth et al., in the abnormal type, α helix is 36% and β sheet is 7%. Therefore, the model by Korth et al. only accounts for a part of the results of CD spectrum and FT-IR. In view of this, the present inventors ~~thought~~ theorized a structure in which α helix is decreased and β structure is increased. According to the prediction of secondary structure by SST, the contents of the structures in the secondary structure are: α helix 5.6%, and β structure 18%. Aside from the precision of the prediction of the secondary structure, prion protein is a protein which likely adopts β structure rather than α helix. Looking into more detail, about the half of the α helix 2 of the normal type is judged as β structure and the other half is C structure (random coil). In α helix 3, except for the residues which are judged as constituting α helix in the latter half thereof, the other regions are in β structure or C structure. Thus, the present inventors made a model that the former half of α helix 2 and the latter half of α helix 3 changed into β structure (model 2).

Please amend the paragraph at page 9, lines 4-9 as follows:

Although the above-described peptide may be used as it is as an immunogen, it is preferred to bind the peptide to a carrier molecule such as keyhole limpet hemocyanin (KLH) or bovine serum albumin (BSA) because the ~~antigenecity~~ antigenicity of the immunogen is increased. Further, it is preferred to use an immunogen in which a plurality of kinds of the above-described peptide are bound to a single carrier molecule.

Please amend the paragraph at page 9, lines 10-19 as follows:

The monoclonal antibody according to the present invention may be prepared by a conventional method except that the above-described immunogen is used. That is, an animal is immunized with the above-described immunogen, and hybridomas derived from antibody-producing cells of the immunized animal are prepared. The hybridomas are screened for those producing monoclonal antibodies which react with the abnormal type prion and do ~~does~~ not substantially react with the normal type prion, and the desired monoclonal antibody is recovered from the screened hybridoma. The method for preparing hybridomas by fusing

antibody-producing cells such as spleen cells and lymphocytes with immortalized cells such as myeloma cells is well-known in the art.

Please amend the paragraph at page 13, lines 22-23 as follows:

11. The resultant was reacted with diluted ~~ABC~~ ABC ® reagent (commercially available from Vector Laboratories, Inc. at Burlingame, CA, USA) at room temperature for 30 minutes.